EXPERT REVIEW OF MOLECULAR DIAGNOSTICS
2020, VOL. 20, NO. 5, 497–508
https://doi.org/10.1080/14737159.2020.1743688

Taylor & Francis
Taylor & Francis Group

REVIEW

Bladder neoplasms and NF-κB: an unfathomed association

Charles Emmanuel Jebaraj Walter [a*], Sankari Durairajan[a*], Kalaiselvi Periyandavan [b], George Priya Doss C[c], Dicky John Davis G[a], Hannah Rachel Vasanthi A[d], Thanka Johnson[a] and Hatem Zayed[e]

[a]Department of Biotechnology, Faculty of Biomedical Sciences, Technology and Research, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Chennai, India; [b]Department of Medical Biochemistry, Dr. ALM PG Institute of Basic Medical Science, University of Madras, Chennai, India; [c]Department of Integrative Biology, School of Biosciences and Technology, VIT University, Vellore, India; [d]Department of Biotechnology, School of Life Sciences, Pondicherry University, Pondicherry, India; [e]Department of Biomedical Sciences, College of Health Sciences, Qatar University, Doha, Qatar

ABSTRACT

Introduction: Bladder cancer is the second most common genitourinary tract cancer and is often recurrent and/or chemoresistant after tumor resection. Cigarette smoking, exposure to aromatic amines, and chronic infection/inflammation are bladder cancer risk factors. NF-κB is a transcription factor that plays a critical role in normal physiology and bladder cancer. Bladder cancer patients have constitutively active NF-κB triggered by pro-inflammatory cytokines, chemokines, and hypoxia, augmenting carcinogenesis and progression.

Areas covered: NF-κB orchestrates protein interactions (PTEN, survivin, VEGF), regulation (CYLD, USP13) and gene expression (Trp 53) resulting in bladder cancer progression, recurrence and resistance to therapy. This review focuses on NF-κB in bladder inflammation, cancer and resistance to therapy.

Expert opinion: NF-κB and bladder cancer necessitate further research to develop better diagnostic and treatment regimens that address progression, recurrence and resistance to therapy. NF-κB is a master regulator that can act with or on minimally one cancer hallmark gene or protein, leading to bladder cancer progression (Tp53, PTEN, VEGF, HMGB1, CYLD, USP13), recurrence (PCNA, BcL-2, JUN) and resistance to therapy (P-gp, twist, SETD6). Thus, an understanding of bladder cancer in relation to NF-κB will offer improved strategies and efficacious targeted therapies resulting in minimal progression, recurrence and resistance to therapy.

ARTICLE HISTORY
Received 20 September 2019
Accepted 13 March 2020

KEYWORDS
Bladder cancer; chemoresistance; inflammation; NF-κB; signal transduction

1. Introduction

Bladder cancer is the second most common genitourinary tract malignancy and the ninth most common cancer worldwide. It is the third leading cause of death related to genitourinary tumors. There is a male predominance of bladder cancer, where men are at higher risk than women in industrialized nations. Over the past few decades, a high prevalence of bladder cancer has been detected in the western world, particularly in North American and European countries. At present, lifestyle changes and increasing awareness of the risks related to bladder cancer have instigated a declining trend in the prevalence rates in these countries. Conversely, industrialization and lifestyle changes in developing countries have increased the prevalence of bladder cancer over the years in Asian countries like India [1,2]. In India, the risk of older males presenting with bladder cancer is more prominent (8.6 males:1 female) [3] than that in western countries (3 males:1 female) [4]. Although comparatively fewer female patients are diagnosed with bladder cancer, their stage at diagnosis is much higher and they have poorer prognoses compared with their male counterparts [5]. Epidemiological studies have stated that intrinsic sex-related differences are a cause for the higher incidence of bladder cancer in men than in women [6,7].

There are many risk factors associated with bladder cancer incidence that can be broadly categorized into environmental, infection/inflammation and/or molecular determinants. Environmental/lifestyle/occupational exposure to compounds, like tobacco or cigarette smoking, aromatic amines, polycyclic hydrocarbons, anilines, nitrates/nitrites, acrolein, coal and arsenic, can trigger bladder carcinogenesis. Tobacco or cigarette smoking is a major risk factor associated with bladder cancer, among others, due to the oxidative stress caused by the exposure leading to inflammation and proliferation [8]. Thus, smoking increases the chance of bladder cancer in men, with an average age at the time of diagnosis of ~ 65 years or older [9]. Chronic inflammation due to pelvic irradiation, indwelling catheters or infection of the bladder lining by *Schistosoma haematobium* causing schistosoma also triggers bladder cancer. Apart from the above, the genetic make-up of some of the population predisposes them to a higher risk of bladder cancer. The molecular determinants are mutations, polymorphisms in oncogenes (TP63, EGFR, Ras, p21), tumor suppressor genes (TP53, Rb1, histidine triad gene) and others (CABLES, Ki67, cyclin D1) [6]. These mutations that occur in certain populations not only increase the risk of bladder cancer but also determine the treatment response of some bladder cancer patients [2].

CONTACT Charles Emmanuel Jebaraj Walter cejwalter@sriramachandra.edu.in Department of Biotechnology, Faculty of Biomedical Sciences, Technology and Research, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Chennai 600116, India
*Share first authorship

Article Highlights

- Bladder inflammation, cancer, and resistance to therapy are associated with NF-κB expression, highlighting the underlying mediation by NF-κB.
- Malignant bladder cancer cells retain constitutively active NF-κB to modulate regulatory mechanisms, such as the down-regulation of p53 and deubiquitinases, among others, enriching the tumor microenvironment with cytokines and VEGF to assist cancer progression.
- Transcription factor NF-κB is able to interact with at least one protein (VEGF, HMGB1, JUN, survivin) or gene (miR130b, PTEN, USP13, CYLD) associated with cancer.
- NF-κB promotes recurrence and resistance to therapy in bladder cancer patients with inherent heterogeneity by deregulating SETD6, P-gp and Twist, among others.

There are many factors that impact bladder cancer initiation, progression, metastasis and treatment response. NF-κB is one of the many crucial factors underlying bladder cancer pathogenesis. This association is drawn from a few reports on the associations of NF-κB in bladder neoplasms. Understanding bladder pathology and the associated molecular factors is important for identifying the prevailing lacunae in bladder cancer pathogenesis. The involvement of NF-κB in bladder cancer progression has been reported in a few pathology studies [10–13], which have helped to reveal the molecular factors responsible for the presence of constitutively active NF-κB in bladder cancer. There are scenarios in which NF-κB also plays a role in the treatment response and resistance in some bladder cancer patients [14,15]. This review provides a compilation of the many mysteries related to the associations of NF-κB and bladder inflammation, malignancy, treatment and chemoresistance.

Histologically, the bladder wall consists of three layers: mucosa, muscularis propria and adventitia, with the serosa/peritoneum at the dome. The mucosa is subdivided into the urothelium, lamina propria, and discontinuous muscularis mucosa. The bladder urothelium is composed of the epithelium (urothelium) that lines the renal pelvis, ureter, bladder, and most of the urethra, with the exception of the distal urethra. The urothelium can be further categorized as superficial (single layer of umbrella cells protecting the underlying cells from urine exposure), intermediate and basal urothelial cells. The lamina propria is located between the mucosal basement membrane and the muscularis propria composed of loose to dense connective tissue, blood vessels, lymphatic vessels and adipose tissue. The discontinuous muscular mucosa is usually associated with intermediate-sized blood vessels, and only 5% of bladders have a well-developed continuous muscularis mucosa. The muscularis propria consists of inner longitudinal, circular and outer longitudinal layers of thick muscle bundles [16] (Figure 1).

2. Bladder inflammation

At the end of the 19th century, the relationship between inflammation and cancer was an expanding focus of research. Inflammatory conditions that occur before malignant changes are observed serve as one of the many probable triggers for some type of cancers. Conversely, an oncogenic change creates an inflammatory microenvironment that protects the progression of tumors in other types of cancer [17]. Inflammation is a self-limiting step, during which the cells are rescued from death due to damage caused by external factors or infection. Interstitial cystitis, proliferative cystitis (von Brunn's nests), chronic cystitis, cystitis cystitica, cystitis glandularis, eosinophilic cystitis and follicular cystitis are a few inflammatory conditions that occur in the bladder (Figure 1). Interstitial cystitis (bladder pain syndrome) manifests suprapubic pain related to bladder filling, accompanied by an increased frequency of urination and other symptoms, with no urinary infection or other pathology. This condition results in classic inflammatory-type lesions that might appear microscopically with normal histology, mucosal ulceration, overlying fibrinous exudates and necrotic debris. The lamina propria is filled with chronic inflammatory cells, prominent mast cells, fibrosis and an absence of bacteria. There is a non-ulcer type of interstitial cystitis where the urothelium appears normal or can have multiple glomerulations [18]. The presence of von Brunn's nests is observed in 85–95% of bladders, increasing with age. The nests consist of cytologically benign urothelium in the lamina propria with regular spacing extending to the same horizontal level at the base of proliferation. They sometimes extend with surface epithelium with minimal inflammation lacking a stromal reaction. The nested variant of urothelial carcinoma is mimicked in florid cases with no muscle invasion, which is usually mistaken for inverted papilloma, carcinoid tumors, paraganglioma and normal paraganglionic cells [19]. Cystitis glandularis is the transformation of mucosal cells lining the urinary bladder where they undergo glandular metaplasia (irritated tissues take on a gland form). These lesions are usually small microscopic foci that occasionally form raised intramucosal or polypoid lesions contained within

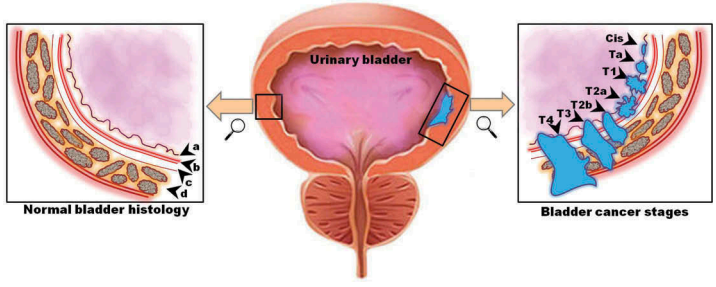

Figure 1. **Bladder histology, inflammation and cancer**. Normal bladder histology (a) Transitional epithelium, (b) Basement membrane, (c) Lamina propria, (d) Muscularis propria. Bladder cancer stages Cis) Carcinoma in situ, Ta) non-invasive papillary tumor, T1) Tumor invading the lamina propria, T2a) Tumor invading superficial muscle, T2b) Tumor invading deep muscle, T3) Tumor extending beyond bladder wall, T4) Tumor invading adjacent organs/structures.

the submucosa. They normally tend to bud from the surface mucosa and merge with von Brunn's nests within the lamina propria and submucosa. Cystitis glandularis can be a non-mucinous and mucinous (intestinal) type. Individuals with diffuse intestinal-type cystitis glandularis are at an increased risk of developing bladder cancer. Like cystitis glandularis, cystitis cystica is a lesion arising from von Brunn's nests but with a degenerated central cystic area [20].

Chronic inflammation is a risk factor in many epithelial cancers, including bladder cancer [21]. Patients with primary superficial bladder carcinoma and some types of invasive bladder carcinoma with an inflammatory reaction experience fewer recurrences and less cancer-related death compared to those with tumors that lack inflammation [22]. High frequencies of inflammation and angiogenesis are associated with invasive bladder tumors [23]. The increased vascular density with an increasing degree of inflammation is an independent indicator of a good prognosis [22]. Estimates of angiogenesis were found to be associated with inflammation since the vascular densities increase with an increasing degree of inflammation, in which both carcinoma and non-neoplastic cells were identified as contributors of VEGF to stimulate angiogenic processes [24]. The molecular pathways of cancer-related inflammation are currently being unraveled, resulting in the identification of new target molecules that may improve diagnosis and treatment.

3. Bladder cancer types

Histopathological classifications of bladder cancer based on the World Health Organization (WHO) are papilloma, papillary carcinoma, bladder neoplasm of low malignant potential, low-grade carcinoma, and high-grade carcinoma [25,26,6]. Bladder cancer T staging by the invading tumor is designated as Ta, T1, T2, T3 and T4. The non-muscle invasive tumor confined to the epithelium and lamina propria mostly belongs to the Ta and T1stages. The muscle invasive tumors invading the detrusor smooth muscle or beyond belong to the T2 to T4 stages [16]. Superficial bladder tumors are non-invasive, protruding papillary tumors that arise from the transitional cells of the mucosal epithelium. These tumors are often recurrent, localized, and non-metastatic; hence, they can be surgically resected. The non-papillary, solid and invasive tumor types usually invade the bladder wall and are highly metastatic [9]. Altogether, approximately 90% of bladder cancers are transitional cell carcinomas, 5% are squamous cell carcinomas (SCCs) and about 2% are adenocarcinomas. However, at the time of presentation, 70% of all bladder diseases in most patients will be superficial (contained in the mucosa, submucosa and/or lamina propria), of which 60–70% will recur after endoscopic resection of the bladder. Further, 20% to 30% of patients will experience disease progression to a higher stage or grade with muscle invasion and/or metastasis (Figure 1).

The complexity and aggression of bladder cancer are due to the use of different oncogenic pathways that take advantage of the cell's physiological location. Thus, the Ta stage of bladder cancer displays a rare occurrence of gene amplification, unlike muscle invasive bladder cancer. Similarly, upstream genetic deletions, such as mutations in TP53, PTEN and RB1, are common in muscle invasive bladder cancer; additionally, hypermethylation of the promoters indicates bladder cancer progression [9,27]. Further, malignant cells utilize the PIK3-mTOR pathway, inducing mutations in genes involved in chromatin remodeling to progress to the invasive form. These pathways and mutations facilitate bladder cancer progression [28].

The urothelium is composed of three cell populations: 90% of the cell population is made up of cytokeratin 5 (CK5)-expressing cells residing in the basal layer; 5% is made up of intermediate cells residing in the suprabasal layer and 5% is made up of superficial cells residing in the luminal layer. Astudy has demonstrated that papillary lesions are formed by intermediate cells and that CK5-expressing basal cells cause carcinoma in situ (CIS) and invasive lesions in muscle and urothelium [7]. The loss of one *Trp53* (tumor suppressor gene encoding p53 protein) allele is more often observed in CIS and muscle invasive disease than in SCC, suggesting the important role played by the gene in the progression of disease to the muscle invasive form. The invasive potential of CIS bladder cancer and papillary lesions differs to a greater extent, potentially due to the physiological location of the cancerous cells in the urothelium. Although the above factors are known to influence bladder cancer, the prerequisite trigger that can set the stage for bladder carcinogenesis is still being debated [7].

4. Nuclear factor-kappa B (NF-κB)

4.1. NF-κB proteins

In 1986, Ranjan Sen and David Baltimore discovered NF-κB in the nucleus, bound to a promoter of the immunoglobulin κ-chain in B cells [29]. NF-κB is a transcription factor that is composed of the following proteins: RelA (p65), RelB, c-Rel, NF-κB1 (p105; p50) and NF-κB2 (p100; p52), as depicted in Figure 2. With these protein members, NF-κB responds to many stimuli like oxidative stress, hypoxia, inflammation, lipopolysaccharide (LPS), proinflammatory chemokines, innate immunity receptors, antigen receptors and environmental factors [30,31]. Each specific stimulus starts a cascade of events leading to a special response in a cell via the classical/canonical pathway (RelA/p50), the non-canonical/alternate pathway (RelB/p100) or the atypical pathway (NEMO/ATM). The classical and alternate pathways are the main pathways, which are distinct and evolutionarily conserved [32,33].

Biological functioning of NF-κB requires dimerization, activation and translocation to the nucleus for gene expression. Dimerization of the Rel proteins is an important step for eliciting NF-κB functions, and all members of the family except RelB can form homo- and heterodimers with one another. The most common NF-κB dimer found in cells is RelA/p50 or RelA/p52. RelB expression is restricted to the thymus, lymph nodes and Peyer's patches, whereas c-Rel is restricted to hematopoietic cells and lymphocytes. Rel proteins are kept inactive in the cytoplasm by specific inhibitory proteins (IκBs), excluding NF-κB1 and NF-κB2, which are processed from the precursor form to the active form [34]. The seminal event in the activation of NF-κB is the phosphorylation of IκBs by IKKs (IκB kinases). The IKKs are the kinases that remove the inhibitory IκB masking the nuclear localization signal sequence (NLS) of NF-κB dimers. Activation of NF-κB relies on the decisive IKK complex, which converges

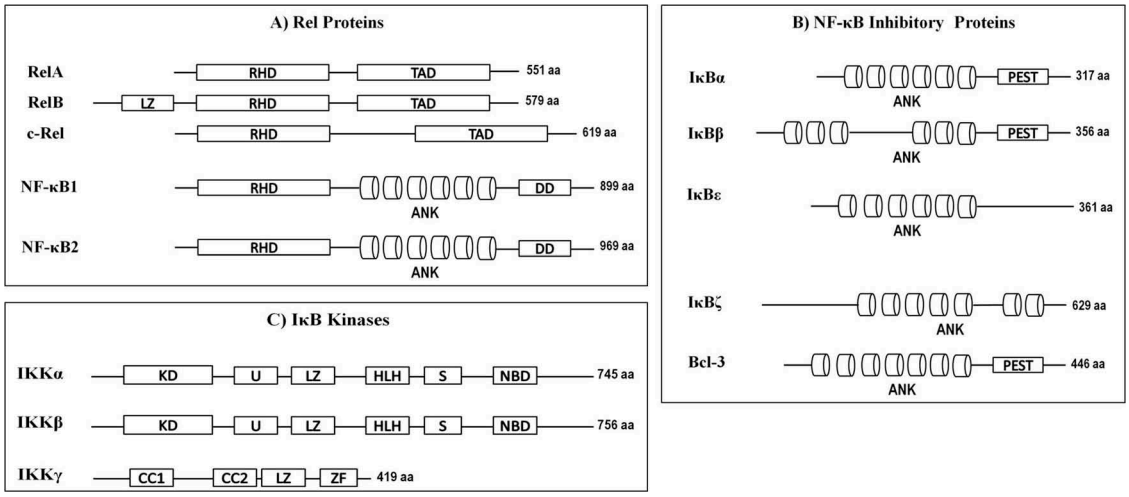

Figure 2. **Proteins of NF-κB family**. (a) Rel proteins: These proteins are characterized by the Rel Homology Domain (RHD). The N-terminal is for DNA binding and it is phosphorylated for regulation, C-terminal is for dimerization and contains the Transactivation Domain (TAD). RelB requires the Leucine Zipper (LZ) region additional to TAD for activation. NF-κB1 and NF-κB2 has Ankyrin Repeat domain (ANK) along with Death Domain (DD) at the C-terminal for inhibitor interactions. (b) NF-κB inhibitory proteins: These proteins retain the NF-κB proteins inactive inside the cytoplasm with Ankyrin Repeats and PEST domains. (c) IκB Kinases: Kinase Domain (KD), U, LZ, Helix Loop Helix (HLH), S and the NEMO Binding Domains (NBD) are features shared by IKKα and IKKβ proteins. IKKγ contains CC1, CC2, LZ and Zinc Finger (ZF) motifs.

according to an array of stimuli. The biochemical function of IKKα and IKKβ appear to be similar, but genetic analysis has shown that they are distinct [35–37]. The activation of IKKs is a tightly regulated process involving several adaptor proteins (TRAFs, IRAKs, RIP proteins and others) [38]. Post-activation, NF-κB dimers translocate to the nucleus to transcribe more than 200 genes related to inflammation, cytokine secretion, chemokine secretion, cell cycle regulation, and angiogenesis, among others, eliciting a response [39]. The regulation of NF-κB proteins is a crucial step and is executed stringently. NF-κB regulates the transcription of p52, RelB and c-Rel belonging to its family [40,41]. Expression of the NF-κB regulators (IκBα, IKKα, IKKβ) is controlled by RelA [42]. The p50 homodimers bind the consensus κB sites on DNA to repress transcription, although the association with Bcl-3 can cause transcriptional activation [43]. Deregulation of NF-κB results in diseases like inflammatory bowel disease, rheumatoid arthritis and cancer.

4.2. NF-κB pathways

The canonical pathway is responsible for functions like inflammation, proliferation, survival, stress responses and pathogen invasion. These functions are delivered to a cell by the transcription of respective target genes of pro-inflammatory cytokines like TNFα, IL-1, IL-6, IL-11 and cell survival factors like Bcl-xL, survivin and Hsp70 [44]. The classical NF-κB pathway regulates the expression of cIAPs, which in turn promotes signal transduction to inhibit caspase activation for cell survival. Cellular uptake or elimination of drugs is determined by NF-κB, which regulates genes that are required for this function [34,45]. It is an important feature that contributes to NF-κB-related chemoresistance in some patients (Figure 3).

The non-canonical pathway is responsible for physiological functions like humoral immunity, peripheral lymphoid organogenesis, cell survival, differentiation, chemokine gene expression, hematopoietic stem cell self-renewal, B cell generation and maintenance and DC functional activation. Non-inflammatory signals triggered by BAFFR, CD40, RANK, and LTβR, among others, activate the alternate pathway [46]. NIK is the central regulator of RelB/p52, where its stabilization and elevated levels are critical for the active pathway [47]. Activities involving activated RelB/p52 are a NEMO-IKK complex independent, slow, steady, persistent activation that is sustained for many hours or days [43,48–51]. Regulation of this pathway occurs via homodimerization of the processed p52 subunits, in which they bind to the κBs site to act as transcriptional repressors (Figure 3).

The atypical pathway is responsible for constant shuttling of NEMO-ATM to generate the constitutively active classical NF-κB dimer. Genotoxic stress activates the NEMO subunit and translocation to the nucleus, where it is sumoylated and subsequently ubiquitinated. This process is carried out by ataxia telangiectasia mutated checkpoint kinase (ATM), tyrosine kinase or casein kinase-2, which later shuttles back to the cytosol to activate IKKβ [52]. The main dimer that participates in this pathway consists of NEMO/ATM proteins. The atypical pathway is NF-κB independent and IKK dependent. The need for NEMO-ATM shuttling to generate the constitutively active classical NF-κB dimer in normal physiology and disease conditions remains unknown (Figure 3).

4.3. The link

The canonical and non-canonical pathways may seem to be independent, but they are interlinked [53]. The TNF-R-associated factors (TRAFs) are key intermediates in both the canonical and non-canonical pathways. TRAF3 has a major role in the non-canonical pathway and is the negative regulator of TRAF2 and TRAF6 in the canonical pathway. In normal and cancer conditions, both NF-κB pathways are co-activated when proteins of both pathways form heterodimers to induce the desired effect. For instance, regulation of RelB transcription is governed by canonical NF-κB dimers, and an association of RelB:p50 subunits during the activation of bone

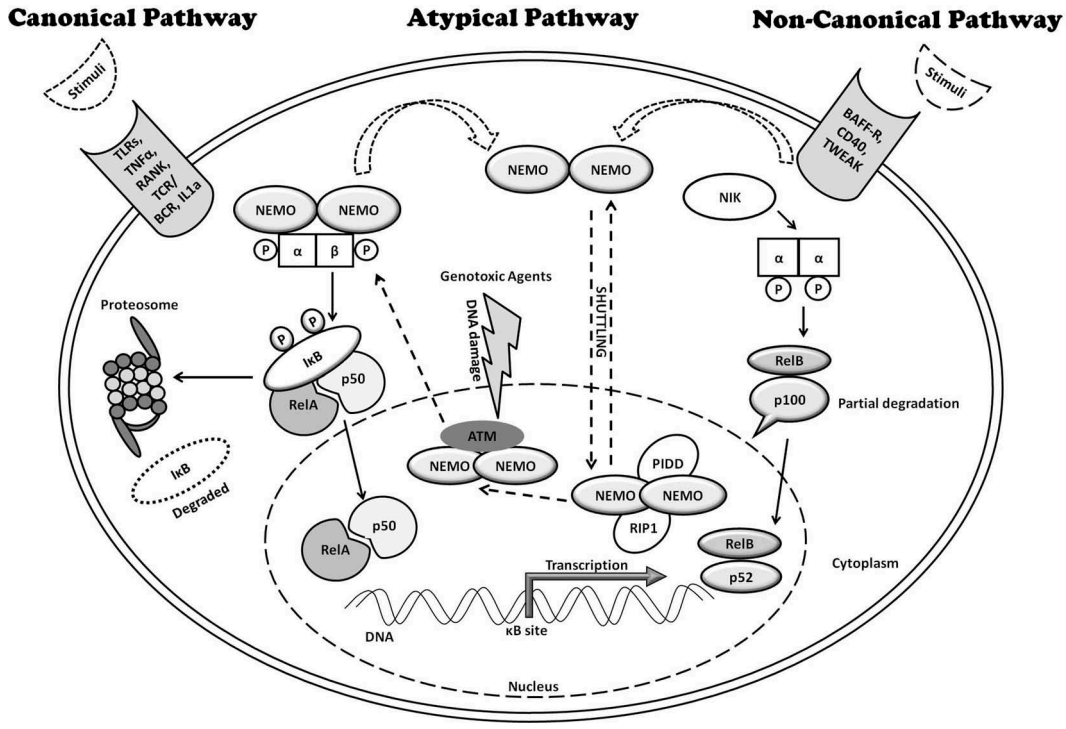

Figure 3. **NF-κB pathways. Canonical pathway**: Specific stimuli trigger respective receptors leading to the activation of NEMO complex. Further, inhibitory complex is degraded to release the active NF-κB protein translocation to the nucleus for gene transcription. **Atypical pathway**: The balance between the nuclear and cytoplasmic NF-κB (RelA/p50) is maintained by the constant shuttling of the NEMO complex with other proteins feeding this pathway. There are other functions of NEMO protein inside the nucleus that are yet to be unraveled. **Non-Canonical pathway**: This pathway specific stimulus triggers the receptors leading to the activation of p100 protein for partial degradation, thus releasing the active NF-κB (RelB/p52) protein to be translocated into the nucleus for gene transcription.

marrow dendritic cell has been reported [54]. These observations indicate that both pathways are part of the development, survival, and functional activation of lymphocytes, dendritic cells and macrophages. They are also part of hematological malignancies in which p52 and RelB have multiple yet distinct effects on the expression of key regulators of the cell cycle, ROS generation and protein stability. All these phenomena highlight the unique mechanisms by which NF-κB functions in disease pathogenesis and cancer (Figure 3).

5. NF-κB in bladder inflammation

Among all bladder cancer risk factors, inflammation is the second most common predisposing factor after cigarette smoking [2] (Figure 4). Progressive inflammation recruits inflammatory cells for cytokine, chemokine, growth factor and angiogenic factor secretion, resulting in the accumulation of free radicals that lead to carcinogenesis, in which the stroma of bladder cancer tissues exhibits inflammatory infiltrates unlike the normal urothelium. The expression of NF-κB in all inflammatory conditions of the bladder is curtailed by only very few studies examining interstitial cystitis and chronic cystitis with NF-κB. It will be interesting to examine the roles of other NF-κB family proteins apart from RelA in bladder inflammation, malignancy and resistance to therapy.

5.1. NF-κB in interstitial cystitis

NF-κB regulates the gene expression of adhesion molecules that recruit inflammatory cells (neutrophils, eosinophils and T lymphocytes) to the site of inflammation in most chronic inflammatory diseases [55,56]. In interstitial cystitis, mast cell activation and an augmented expansion in its numbers are implicated in disease progression. Increased expression of RelA in the nucleus of epithelial cells and submucosal layers of interstitial cystitis patients has been observed, while normal urothelial cells show cytoplasmic and perinuclear immunostaining for RelA [57]. Patients with interstitial cystitis show increased submucosal activation of RelA, suggesting stimulation by extracellular stimuli, which have not been observed in normal samples [58]. These findings clearly underscore the potential role of NF-κB in orchestrating and maintaining inflammatory and immune responses in patients with interstitial cystitis, which ensue after the initial stimulation, suggesting a correlation between the activation of NF-κB and the pathophysiology of this disease.

5.2. NF-κB in chronic cystitis

NF-κB is activated by upstream lymphotoxin β receptor (LTβR) in chronic cystitis and potentiates its progression to bladder cancer [59,60]. LTβR belongs to the tumor necrosis factor (TNF) receptor superfamily expressed on epithelial cells and most other cells [21]. LTβR signaling is involved in inflammatory processes and carcinogenesis, especially inflammation-induced carcinogenesis [61,62]. Astudy assessing chronic cystitis, bladder cancer and healthy mucosa tissues showed that along with LTβR, both NF-κB:canonical RelA and non-canonical RelB were expressed at higher levels in chronic cystitis and bladder cancer than in healthy bladder mucosa. In addition, the alternate pathway protein RelB predominantly potentiates the transition from bladder

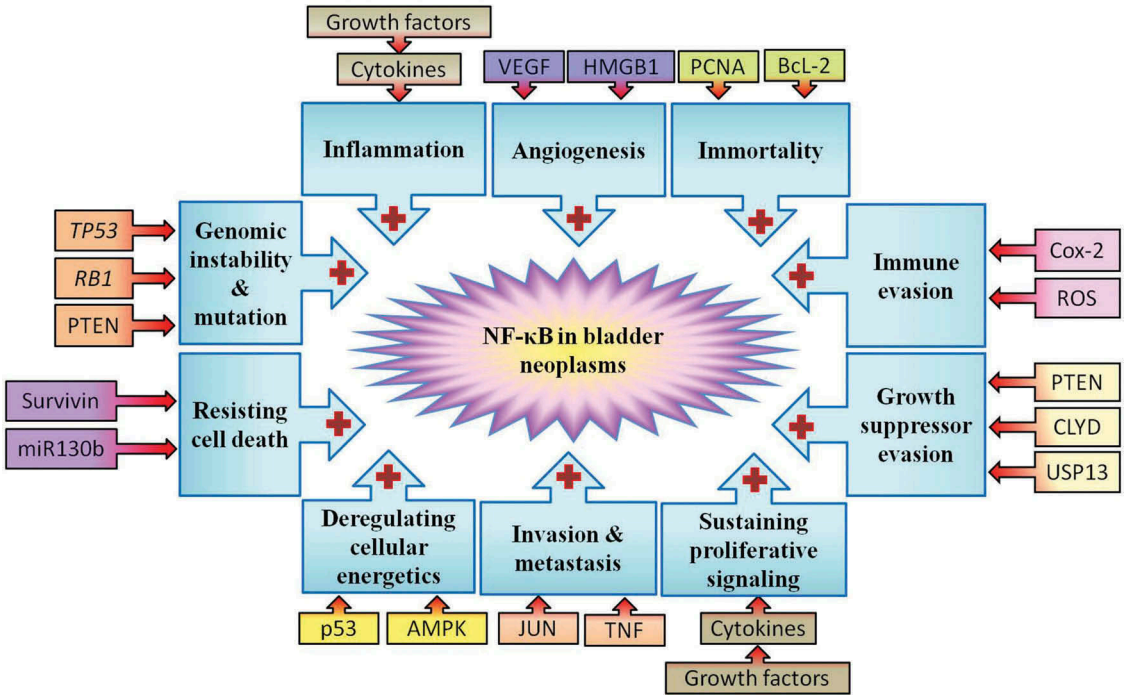

Figure 4. **NF-κB and bladder cancer**: All the cancer hallmarks [115] are met by the NF-κB in bladder cancer and treatment. The contribution to each of the hallmark is by a variety of agents like enzymes, growth factors, cytokines, microRNAs, etc. The mechanism by which NF-κB involves these agents in bladder carcinogenesis, progression, recurrence and chemoresistance are the focus of this review.

inflammation to cancer. The study concluded that there was no significance between age or gender and the expression of LTβR, RelA and RelB [21]. Thus, chronic cystitis exhibiting active NF-κB pathways might lead to bladder cancer via inflammation-associated proteins like LTβR.

6. NF-κB in bladder cancer

Localization of the NF-κB subunit by immunohistochemical staining reveals the active role played by the protein in bladder cancer. Nuclear expression of the RelA subunit of NF-κB increases with increasing tumor grade and T-category [11]. Differential nuclear expression of the NF-κB subunits RelA/p50 when assessed together has been observed by immunohistochemistry [10]. Similarly, in muscle-invasive bladder cancers, NF-κB activation, as indicated by nuclear RelA subunit expression, is positively associated with the tumor histological grade, T-category and chemoradiation resistance [14]. Overexpression of the RelA subunit could be an initiating event, functioning as a by-product of another signaling cascade and collaborating with another protein to induce a stimulus or regulatory mechanism. The interactions ultimately result in aberrant NF-κB activation, leading to bladder cancer progression.

Deciphering the mechanism responsible for the malignant transformation of bladder cells is crucial because bladder cancer patients often have a poor prognosis and survival due to progression, recurrence, metastasis and resistance to therapy. Many molecules, like STAT3 [63], AP-1 [63], HIF-1 [64], survivin [14], deubiquitinases (DUBs) [12,65,66], nuclear proteins [67], microRNAs [12], and nitric oxide (NO) [68], among others, are associated with NF-κB to induce the aforementioned clinical implications. Similarly, oncogenic signals activate several transcription factors like AP-1 and STAT3, which share common genetic targets with NF-κB. Hence, during tumorigenesis, premalignant cells induce NF-κB, AP-1 and STAT3 transcription factors to collaborate and promote cancer progression [63]. Such strong NF-κB signaling can induce other transcriptional complexes like HIF-1α (induced during hypoxia), which accumulates even when the cell is normoxic [64]. NF-κB increases the expression of survivin (IAP family member) – a downstream molecule that in turn mediates NF-κB signaling to facilitate the progression of bladder malignancies and drug resistance [14]. High mobility group box 1 (HMGB1) is a non-histone nuclear protein that is overexpressed in bladder cancer. Knockdown of HMGB1 expression significantly inhibits the expression levels of RelA and VEGF-C (a downstream target gene of NF-κB activation), up-regulates IκBα expression, and suppresses the nuclear translocation and DNA-binding activity of RelA [69]. HMGB1 regulates VEGF-C expression during the development of bladder cancer *via* the NF-κB signaling pathway [67]. Thus, NF-κB can collaborate, induce and mediate oncogenic signaling via other proteins in the cell.

Deregulation of NF-κB signaling is a common event in chronic inflammation and tumorigenesis despite stringent regulation [70–72]. Regulatory modes are governed by multiple feedback loops, tumor suppressors (phosphatase and TENsin homolog [PTEN], cylindromatosis [CYLD]), regulators (deubiquitinases [CYLD], ubiquitin-specific protease 13 [USP13]) and microRNAs [12,65,66]. Ubiquitination is also an important step in NF-κB activation, and bypassing this regulatory step results in constitutive activation [73,74]. PTEN gene mutation or protein downregulation initiates superficial papillary transitional cell carcinoma (TCC) and promotes

progression of CIS to advanced tumors of the bladder [66,75]. Similarly, CYLD is a DUB, a tumor suppressor and also a negative regulator of NF-κB [76]. The microRNA miR-130b (involved in cell cycle control, the EMT process, promoting cancer cell proliferation, invasion and migration) is overexpressed, and NF-κB up-regulates its expression for persistent activation and progression of bladder cancer [14,65]. When the NF-κB/miR-130b/301b cluster axis is promoted, it leads to the loss of PTEN and CLYD expression by decreasing USP13 expression to facilitate bladder cancer progression [66]. Thus, NF-κB hosts the interplay of DUB repression to surpass the regulatory mechanism by overexpressing oncogenic miR-130b in bladder cancers. Apart from the tightly regulated mechanisms involving DUBs, there is another layer of regulation of the IκB degradation imposed by post-translational modifications like phosphorylation, acetylation, methylation and ubiquitination. These modifications act to suppress activated NF-κB in bladder cancer and are elaborated in detail elsewhere [12].

Molecules like nitric oxide [68], ursolic acid [77] and cigarette smoke [78,79] can induce NF-κB in bladder cancer. Nitric oxide is secreted by inducible NO synthase (iNOS), and the mechanisms of tumor invasion in bladder cancer are still unknown. NO can possess both pro- and anti-tumorigenic activity; however, 50% of bladder cancer patients are positive for iNOS expression, with tenacity for early recurrence and invasive progression, unlike the normal urothelium [68]. Sustained iNOS activity is achieved by constitutively activated NF-κB, and cells lacking NO production are sensitive to the cytotoxic effects of external NO [80,81]. Prolonged iNOS activity causes DNA damage, increased cell proliferation and tumor vascularity in bladder cancer [68]. Ursolic acid is a functional triterpene that is capable of modulating the Akt/NF-κB pathways responsible for anti-apoptosis and drug resistance in bladder cancer [15]. Ursolic acid influences the Akt/NF-κB pathway by repressing Bcl-2 and IκBα phosphorylation and modulating reactive oxygen species (ROS), AMP-activated protein kinase (AMPK) to induce apoptosis [77]. The major risk factor for bladder cancer is tobacco or cigarette smoking, and a cigarette smoke extract (CSE) version of the same can induce cell proliferation in normal human urothelial cells (SV-HUC-1) via an unknown mechanism [78,79]. Aplausible mechanism involves NF-κB, in which it increases IKK phosphorylation and reduces IκB expression for activation and co-activation of the MAPK/AP-1, ERK1/2 and JNK pathways. CSE modulates functions like the cell cycle distribution, increased expression of PCNA (proliferating cell nuclear antigen), cyclin D1 and decreased expression of p21, which are the steps of cellular proliferation and main targets of NF-κB [82]. CSE-induced cell proliferation of normal cells is prevented with the inhibition of NF-κB [78,79]. Thus, a stimulus of any magnitude or dimension is capable of inducing NF-κB expression in bladder cancer. The factors leading to bladder cancer initiation, progression and metastasis are also responsible for the treatment response. The mechanism by which NF-κB aids or disrupts the treatment response to facilitate resistance in a select group of bladder cancer patients is another mystery that must be solved.

7. NF-κB in bladder cancer chemoresistance

Bladder cancer is a heterogeneous disease and prediction of an accurate prognosis is difficult even when accounting for the European Organization for Research and Treatment of Cancer (EORTC) proposed scoring systems and risk tables for predicting recurrence and progression [83,84]. There are various treatment modalities based on bladder cancer stages, and it is a challenge to identify the therapy non-responder subgroup among patients who are highly susceptible to recurrence and progression. Treatment failure in bladder cancer patients is due to the presence of BCG adjuvant intravesical therapy non-responders, cisplatin non-responders and those patients who initially respond to cisplatin and later develop resistance. Although many molecular markers are being investigated, none to date has been able to address the challenges related to the clinical course and treatment outcomes of bladder cancer. Some notable molecular markers are p53, Rb, cyclin A (cell cycle regulators), IAP and Bcl-2 family proteins (apoptosis mediators) [14,65].

Molecular phenotyping of individual bladder cancers has identified unique molecular differences between patients suffering from the same disease. Papillary tumors and flat, invasive tumors have distinct molecular alterations, especially in cell cycle regulation, a key event determining the behavior of bladder cancer [85]. For example, mutation of the p53 gene is associated with the pathological stage and grade in bladder cancer, which can serve as a single independent predictor of disease progression [86]. Increased nuclear accumulation of p53 predicts a higher recurrence and decreased overall survival, thus establishing itself as a reliable and consistent prognostic marker for bladder cancer progression [87–89]. However, those patients without p53 mutation also encounter bladder cancer progression, which indicates that another pathway is involved in this process, namely, the mutated/altered RB gene. For therapy, patients with altered p53 have increased susceptibility to cisplatin (or other DNA-damaging agents) treatment than those with the wild-type p53 gene [89,90]. Apart from p53 and RB, many aberrantly expressed factors, such as thymidylate synthase (TS), Bcl-2, Cox-2, epidermal growth factor receptor (EGF-R), NF-κB, protein kinase B (PKB or Akt), PTEN, telomerase, coxsackie adenovirus receptor (CAR) and the uroplakin gene (specifically expressed in transitional cell epithelium in bladder cancer), are responsible for chemoresistance in bladder cancer [85]. Despite many contributing factors to bladder cancer, the contribution of NF-κB is significant because it controls cell metabolism/survival [91–93].

7.1. NF-κB partners in bladder cancer chemoresistance

NF-κB post-translational modifications like phosphorylation, acetylation, methylation and ubiquitination affect downstream NF-κB signaling during regulation. For instance, NF-κB methylation at K218 and K221 augments NF-κB target gene transcription, arginine methylation regulates NF-κB-dependent transcription and RelA methylation at lysine 310 modulates

its transactivation properties [12,13]. In a similar regulatory setting, NF-κB is antagonized by p53 to repress carcinogenesis in normal physiology. Interestingly, the NF-κB and p53 pathways are integrated when the promoters and enhancers of target genes are both regulated by p53 and NF-κB to determine cell fate [94]. However, when p53 is mutated, it loses its regulatory hold on NF-κB, which is a common event in invasive bladder cancer. Aberrant p53 methylation is carried out by SET7/9, SMYD2, SETD8, and G9a/GLP at K372, K370, K382, and K373, respectively [13]. Thus, NF-κB has the capacity to modulate the regulatory mechanisms to curb its activity in support of bladder cancer progression and resistance to therapy [95]. Further, it disrupts the enzymes associated with these post-translational modifications like SETD6 (methyltransferase, an epigenetic modulator of NF-κB). SETD6 plays a significant role in contributing to bladder carcinogenesis by regulating NF-κB expression levels. During therapy, BCG administration induces the NF-κB-mediated inflammatory response that is locally regulated by SETD6, which has a propensity to lead to resistance [13].

In high-grade bladder cancer after chemotherapy, the residual tumor shows increased multidrug resistance gene 1 (MDR1) mRNA and P-glycoprotein (P-gp) expressions [96]. P-gp is an ATP-dependent cellular efflux pump encoded by MDR1. Concentrations of chemotherapies (anthracyclines, vinca alkaloids and taxanes) are reduced upon overexpression of P-gp, contributing to chemoresistance [97]. Chemoresistance in bladder cancer is proportional to the aberrant P-gp expression, predicting a poor clinical outcome [98]. Similarly, Twist is a transcription factor that is overexpressed to promote tumor invasion, angiogenesis, survival and chemoresistance in high-grade bladder cancer, predicting a poor prognosis [99,100]. It acts as a regulator of P-gp in bladder cancer cells and it is an evolutionarily conserved target of NF-κB [98]. Like bladder cancer progression, recurrence impacts patient survival. NF-κB has a significant association with bladder cancer progression; however, it is insignificant in terms of recurrence, as confirmed by a study that demonstrated an association of high expression of NF-κB with progression but not recurrence. It also had a negative impact on progression-free survival in this specific patient group. Thus, NF-κB allows the identification of this high-risk subgroup of patients at higher risk for bladder cancer progression in high-grade non-muscle-invasive bladder cancer [101]. These findings emphasize the link between NF-κB and many associated factors in bladder cancer chemoresistance, although many grey areas are yet to be addressed [13].

Based on the stimulus, many pathways interact with NF-κB, and emerging preclinical evidence suggests a role for sex hormone in bladder carcinogenesis and progression. Accordingly, NF-κB and androgen receptor (AR) signaling crosstalk in prostate cancer cells has been suggested, and NF-κB plays a role in AR-induced gemcitabine resistance in bladder cancer cells [102,103]. Male-predominant bladder cancer is influenced by AR signaling, which critically contributes to the gender-based difference in bladder cancer incidence [104]. In patients with prostate and bladder cancer, androgen deprivation therapy (ADT) is used as a treatment, which prevents further development and recurrence of bladder tumors [105,106]. AR-positive bladder cancer growth is inhibited by ADT, in which NF-κB RelA/p50 and tissue transglutaminase form a complex to bind the AR promoter at NF-κB response element sites; thus, NF-κB regulates tumor cell sensitivity to drug-induced apoptosis [103]. The mechanism by which NF-κB regulates cisplatin sensitivity could involve activation of the antiapoptotic bcl-2 family, c-FLIP, c-JUN and histone modifications [107,108]. While NF-κB regulates tumor cell sensitivity to drug-induced apoptosis, it has an opposite effect in the presence of androgens. NF-κB in the presence of androgens reduces cisplatin-induced cytotoxicity in AR-positive bladder cancer cells. Bladder cells that are AR-positive are modulated by RelA from neoplastic transformation and growth, unlike AR-negative cells [103]. Discrepancies as such still prevail regarding the associations of NF-κB in bladder cancer and chemoresistance. BCG immunotherapy results in overexpression of NF-κB1 and influences the immune response [109]. In some patients, an NF-κB gene genetic predisposition toward bladder cancer is observed and has a major contribution to the BCG treatment response [110–112]. Astudy of patients carrying the NF-κB del/del genotype demonstrated a 2.5-fold increased risk of recurrence compared with that of patients with the ins/ins genotype treated with BCG. Further, polymorphisms in the NF-κB promoter, an insertion/deletion ATTG functional polymorphism at – 94, are linked to bladder cancer recurrence [109]. These results emphasize the crucial role of NF-κB genes in determining the treatment response in bladder cancer patients.

Exploring newer agents for the treatment of bladder cancer is warranted since it is well known to develop chemoresistance after a period of time. The application of programmed cell death protein (PD-1) and programmed death ligand 1 (PD-L1) immune checkpoint therapies has launched a new era for bladder cancer treatment; however, the study of critical biomarkers and molecular mechanisms for progression of bladder cancer is still imperative. Treatments targeting NF-κB will be effective in curbing bladder cancer progression and rendering the cells sensitive to chemotherapy. Pre-treatment of bladder cancer cells with 5-azacytidine enhances sensitivity to cisplatin and docetaxel treatment by inducing re-expression of target of methylation-induced silencing 1 (TMS1) protein, which downregulates NF-κB [113]. Further, pre-treatment results in increased cell death after drug combination treatment in which epigenetically silenced genes are up-regulated to induce the desired cell death [114]. Similarly, YM-155 is a new agent that can potently suppress the expression of survivin, inducing apoptosis, cell cycle arrest by inhibiting the tumorigenic NF-κB/survivin pathway in bladder cancer [14,65]. Such agents hold promise to overcome progression, recurrence and chemoradiation resistance.

8. Conclusion

NF-κB has a role in the pathogenesis of bladder cancer since its inception as an organ-contained transitional cell carcinoma that is recurrent and/or chemoresistant in patients. All or most of the predisposing factors, like cigarette smoking, persistent infection and exposure to aromatic amines, lay the foundation for an underlying chronic inflammation that triggers NF-κB. It is interesting to note that NF-κB utilizes many proteins representing cancer hallmarks leading to bladder cancer progression. Since NF-κB is involved in several critical cell survival mechanisms, deregulation by persistent inducing stimuli of

this crucial factor or its upstream molecules or their genes results in bladder neoplasms. Patients with mutations in the Trp53, RB1 and PTEN genes have high genomic instability that leads to bladder cancer. Constitutively active NF-κB creates an inflammatory microenvironment due to aberrant cytokine, growth factor expression. These proliferative signals induce angiogenic factors like VEGF and HMGB1, which equips the malignant bladder cell with the ability to resist cell death by inducing survivin, miR130b, allowing immune evasion by inducing reactive oxygen species, COX2; allowing growth suppressor evasion by inducing or suppressing deubiquitinases like CYLD, PTEN, and USP13; deregulating cellular energetics with assistance from p53, AMPK; and allowing immortality, invasion and metastasis by expressing PCNA, BcL-2, JUN, and TNFs. In bladder cancer, NF-κB plays a role in drug efflux, and active NF-κB in immune cells determines the success rate of therapy across populations. BCG administration induces an NF-κB-mediated inflammatory response that is locally regulated by SETD6, which has the propensity to contribute to chemoresistance in some bladder cancer patients. Genetic polymorphisms, P-gp and twist expression, and the presence of androgens, among others, target NF-κB to influence the treatment response and resistance. NF-κB oppresses all the regulatory systems from genes to proteins to influence bladder cancer progression and the treatment response. Thus, understanding bladder cancer with NF-κB will offer better strategies and efficacious targeted therapies with minimal progression, recurrence and resistance to therapy.

9. Expert opinion

NF-κB is a well-regulated pathway with several regulatory loops that become deregulated to support bladder cancer progression. The relationship between NF-κB and bladder cancer has been established in a few pathology studies [10,11,101]. When considering bladder cancer, it is interesting to note that different bladder cancers, like papillary tumors and invasive tumors, when subjected to molecular phenotyping reveal unique molecular differences among patients suffering from the same disease [14]. The underlying causal molecule or mechanism for the inherent molecular differences in bladder cancer is unknown. Nevertheless, patients diagnosed with bladder cancer are subjected to different treatment modalities based on factors like age, gender, grade, and stage, among others, which impacts patient prognosis and leads to differences in the treatment response in bladder cancer patients undergoing the same treatment. In addition, a subgroup of patients receiving treatment develops resistance (either before or after a few treatment sessions). Identifying this patient subgroup will impact their prognosis and treatment outcomes [14,65].

NF-κB possesses extreme plasticity and utilizes several mechanisms involved in disease pathogenesis. Avenues by which NF-κB is utilized in bladder cancer and its progression is a key research focus. Investigations of the associations of NF-κB with bladder cancer initiation, progression, recurrence, treatment and resistance could lead to realistic implementations in clinical practice. NF-κB, as a cell fate determinant, serves an equivalent role in bladder cancer, where constitutively active NF-κB collaborates with many proteins like p53, JUN, VEGF, PTEN, SETD6, USP13, P-gp, and Twist; enzymes like AMPK and CYLD; and genes like Trp53, RB1, and miRNAs like miR130b, among others to suppress the regulatory mechanisms responsible for its inactivation. By oppressing all the regulatory systems, ranging from genes to proteins, NF-κB is able to influence bladder cancer progression and treatment responses. NF-κB is a cancer hallmark, as stated by Hanahan and Weinbergh [115]. The influence of one transcription factor, NF-κB, along with several others related to the cancer hallmarks pertaining to bladder cancer progression is intriguing.

In bladder cancer, there is a preferential bias between NF-κB subunits during progression and a currently unknown function [10]. Technically, NF-κB exists as a dimer (RelA/p50) that must dimerize to elicit different cell functions based on the specific stimuli. Further, the involvement of various NF-κB subunits in bladder inflammation, carcinogenesis, progression, recurrence, treatment response and resistance in some patients necessitates research. Deciphering the functions and associations of NF-κB in bladder cancer pathogenesis will be a tremendous advancement in the field. The NF-κB transcription factor is able to participate in all cancer hallmark traits, possessing the capability to reverse resistance to therapies, which are key drug targets to improve bladder cancer patients' quality of life and survival.

Acknowledgments

The authors thank Mrs. Vidhya L, Senior Lecturer in English, Faculty of Allied Health Sciences, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), India, for proofreading the manuscript.

Funding

This work was supported by Young Faculty Research award (GATE) funding from Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), India.

Declaration of interest

This work was supported by a Young Faculty Research award (GATE) funded by Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), India to authors CEJW and DJDG. Author SD was awarded a chancellor fellowship from Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), India. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewers' Disclosure

Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.

ORCID

Charles Emmanuel Jebaraj Walter http://orcid.org/0000-0003-1370-5205
Kalaiselvi Periyandavan http://orcid.org/0000-0001-5270-2651

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108.
2. Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: a global overview and recent trends. Eur Urol. 2017;71(1):96–108.
3. Yuvaraja TB, Waigankar S, Bakshi G, et al. Genitourinary cancers: summary of Indian data. South Asian J Cancer. 2016;5(3):122–124.
4. Ray D, Mondal R, Acharya S, et al. a retrospective study of bladder cancer and the impact of age, sex and smoking habits related clinicopathological correlations in tribal population of Bankura, WB, India. IOSR J Dent Med Sci. 2013;10(4):29–32.
5. Chinnasamy R, Krishnamoorthy S, Joseph L, et al. Clinicopathological study of bladder cancer in a tertiary care center of South India and Impact of age, gender, and tobacco in causing bladder cancer: a single center experience. Int J Sci Stud. 2016;3(10):72–77.
6. Kaufman DS, Shipley WU, Feldman AS. Bladder cancer. Lancet. 2009;374(9685):239–249.
7. Van Batavia J, Yamany T, Molotkov a, et al. Bladder cancers arise from distinct urothelial sub-populations. Nat Cell Biol. 2014;16(10):982.
8. Leibovici D, Grossman HB, Dinney CP, et al. Polymorphisms in inflammation genes and bladder cancer: from initiation to recurrence, progression, and survival. J clin oncol. 2005;23(24):5746–5756.
9. Crawford JM. The origins of bladder cancer. Lab Invest. 2008;88(7):686–693.
10. Durairajan S, Jebaraj Walter CE, Samuel MD, et al. Differential expression of NF-κB heterodimer RelA/p50 in human urothelial carcinoma. PeerJ. 2018;6:e5563.
11. Levidou G, Saetta AA, Korkolopoulou P, et al. Clinical significance of nuclear factor (NF)-κB levels in urothelial carcinoma of the urinary bladder. Virchows Arch. 2008;452(3):295–304.

•• **Describes the levels of p65/RelA expression immunohistochemically in urothelial carcinoma.**

12. Mukherjee N, Houston TJ, Cardenas E, et al. To be an ally or an adversary in bladder cancer: the NF-κB story has not unfolded. Carcinogenesis. 2015;36(3):299–306.

• **Describes the lacunae in NF-κB story in bladder cancer.**

13. Mukherjee N, Cardenas E, Bedolla R, et al. SETD6 regulates NF-κB signaling in urothelial cell survival: implications for bladder cancer. Oncotarget. 2017;8(9):15114.
14. Cui X, Shen D, Kong C, et al. NF-κB suppresses apoptosis and promotes bladder cancer cell proliferation by upregulating survivin expression *in vitro* and *in vivo*. Sci Rep. 2017;7:40723.
15. Shanmugam MK, Dai X, Kumar AP, et al. Ursolic acid in cancer prevention and treatment: molecular targets, pharmacokinetics and clinical studies. Biochem Pharmacol. 2013;85(11):1579–1587.
16. Kobayashi T, Owczarek TB, McKiernan JM, et al. Modelling bladder cancer in mice: opportunities and challenges. Nat Rev Cancer. 2015;15(1):42–54.
17. Mantovani a, Allavena P, Sica a, et al. Cancer-related inflammation. Nature. 2008;454(7203):436–444.
18. Hanno P, Nordling J, Fall M. Bladder pain syndrome. Med Clin North Am. 2011;95(1):55–73.
19. Young RH. Tumor-like lesions of the urinary bladder. Mod Pathol. 2009;22(S2):S37–S52.
20. Bastianpillai C, Warner R, Beltran L, et al. Cystitis cystica and glandularis producing large bladder masses in a 16-year-old boy. J R Soc Med Open. 2018;9(3):1–3.
21. Shen M, Duan X, Zhou P, et al. Lymphotoxin β receptor activation promotes bladder cancer in a nuclear factor-κB-dependent manner. Mol Med Rep. 2015;11(2):783–790.
22. Offersen BV, Knap MM, Marcussen N, et al. Intense inflammation in bladder carcinoma is associated with angiogenesis and indicates good prognosis. Br J Cancer. 2002;87(12):1422–1430.
23. Takeuchi H, Tanaka M, Tanaka a, et al. Predominance of M2-polarized macrophages in bladder cancer affects angiogenesis, tumor grade and invasiveness. Oncol Lett. 2016;11(5):3403–3408.
24. ŁP F, Górnicka B. Role of angiogenesis in urothelial bladder carcinoma. Cent European J Urol. 2016;69(3):258.
25. Montironi R, Lopez-Beltran a. The 2004 WHO classification of bladder tumors: a summary and commentary. Int J Surg Pathol. 2005;13(2):143–153.
26. Humphrey PA, Moch H, Cubilla AL, et al. The 2016 WHO classification of tumours of the urinary system and male genital organs—part B: prostate and bladder tumours. Eur Urol. 2016;70(1):106–119.
27. Yates DR, Rehman I, Abbod MF, et al. Promoter hypermethylation identifies progression risk in bladder cancer. Clin Cancer Res. 2007;13(7):2046–2053.
28. Chekaluk Y, Wu C-L, Rosenberg J, et al. Identification of nine genomic regions of amplification in urothelial carcinoma, correlation with stage, and potential prognostic and therapeutic value. PLoS One. 2013;8(4):e60927.
29. Sen R, Baltimore D. Inducibility of κ immunoglobulin enhancer-binding protein NF-κB by a posttranslational mechanism. Cell. 1986;47(6):921–928.
30. Baima ET, Guzova JA, Mathialagan S, et al. Novel insights into the cellular mechanisms of the anti-inflammatory effects of NF-κB essential modulator binding domain peptides. J Biol Chem. 2010;285(18):13498–13506.
31. Zhang Q, Lenardo MJ, Baltimore D. 30 Years of NF-κB: a blossoming of relevance to human pathobiology. Cell. 2017;168(1–2):37–57.

• **Describes the evolution of NF-κB and its functions.**

32. Hoffmann a, Baltimore D. Circuitry of nuclear factor κB signaling. Immunol Rev. 2006;210(1):171–186.
33. Perkins ND. Integrating cell-signalling pathways with NF-κB and IKK function. Nat Rev Mol Cell Biol. 2007;8(1):49–62.
34. Delhalle S, Blasius R, Dicato M, et al. a beginner's guide to NF-κB signaling pathways. Ann N Y Acad Sci. 2004;1030(1):1–3.
35. Israël a. The IKK complex, a central regulator of NF-κB activation. Cold Spring Harb Perspect Biol. 2010;2(3):a000158.
36. Oeckinghaus a, Hayden MS, Ghosh S. Crosstalk in NF-κB signaling pathways. Nat Immunol. 2011;12(8):695–708.
37. Begalli F, Bennett J, Capece D, et al. Unlocking the NF-κB conundrum: embracing complexity to achieve specificity. Biomedicines. 2017;5(3):50.
38. Durand JK, Baldwin AS. Targeting IKK and NF-κB for therapy. Adv Protein Chem Struct Biol. 2017;107:77–115.
39. Brasier AR. The NF-κB regulatory network. Cardiovasc Toxicol. 2006;6(2):111–130.
40. Hinz M, Scheidereit C. The IκB kinase complex in NF-κB regulation and beyond. EMBO Rep. 2014;15(1):46–61.
41. Tsui R, Kearns JD, Lynch C, et al. IκBβ enhances the generation of the low-affinity NFκB/RelA homodimer. Nat Commun. 2015;6:7068.
42. Yang J, Mitra a, Dojer N, et al. a probabilistic approach to learn chromatin architecture and accurate inference of the NF-κB/RelA regulatory network using ChIP-Seq. Nucleic Acids Res. 2013;41(15):7240–7259.
43. Tao Z, Fusco a, Huang DB, et al. p100/IκBδ sequesters and inhibits NF-κB through kappaBsome formation. Proc Nat Acad Sci. 2014;111(45):15946–15951.
44. Bollrath J, Greten FR. IKK/NF-κB and STAT3 pathways: central signalling hubs in inflammation-mediated tumour promotion and metastasis. EMBO Rep. 2009;10(12):1314–1319.
45. Herpers B, Wink S, Fredriksson L, et al. Activation of the Nrf2 response by intrinsic hepatotoxic drugs correlates with suppression of NF-κB activation and sensitizes toward TNFα-induced cytotoxicity. Arch Toxicol. 2016;90(5):1163–1179.
46. Sun SC. Non-canonical NF-κB signaling pathway. Cell Res. 2011;21(1):71–85.
47. Uno M, Saitoh Y, Mochida K, et al. NF-κB inducing kinase, a central signaling component of the non-canonical pathway of NF-κB, contributes to ovarian cancer progression. PLoS One. 2014;9(2):e88347.
48. Sun SC. The non-canonical NF-κB pathway in immunity and inflammation. Nat Rev Immunol. 2017;17(9):545–558.

49. Tapia MA, González-Navarrete I, Dalmases a, et al. Inhibition of the canonical IKK/NFκB pathway sensitizes human cancer cells to doxorubicin. Cell Cycle. 2007;6(18):2284–2292.
50. Zhao C, Xiu Y, Ashton J, et al. ABSTRACT: noncanonical NF-κB signaling regulates hematopoietic stem cell self-renewal and microenvironment interactions. Blood. 2011;118:859.
51. Gasparini C, Celeghini C, Monasta L, et al. NF-κB pathways in hematological malignancies. Cell Mol Life Sci. 2014;71(11):2083–2102.
52. Hoesel B, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer. 2013;12(1):86.
•• **Describes the intricate NF-κB signaling linking inflammation and cancer.**
53. Yu L, Li L, Medeiros LJ, et al. NF-κB signaling pathway and its potential as a target for therapy in lymphoid neoplasms. Blood Rev. 2017;31(2):77–92.
54. Shih VF, Davis-Turak J, Macal M, et al. Control of RelB during dendritic cell activation integrates canonical and noncanonical NF-κB pathways. Nat Immunol. 2012;13(12):1162–1170.
55. Monaco C, Andreakos E, Kiriakidis S, et al. Canonical pathway of nuclear factor κB activation selectively regulates proinflammatory and prothrombotic responses in human atherosclerosis. Proc Nat Acad Sci. 2004;101(15):5634–5639.
56. Westbrook AM, Szakmary a, Schiestl RH. Mechanisms of intestinal inflammation and development of associated cancers: lessons learned from mouse models. Mutat Res/Rev Mutat Res. 2010;705(1):40–59.
57. Abdel-mageed AB, Ghoniem GM. Potential role of rel/nuclear factor-kappa B in the pathogenesis of interstitial cystitis. J Urol. 1998;160(6 Part 1):2000–2003.
58. Abdel-Mageed AB. NF-κB-dependent gene expression of proinflammatory cytokines in T24 cells: possible role in interstitial cystitis. Urol Res. 2003;31(5):300–305.
59. Dhawan P, Su Y, Thu YM, et al. The lymphotoxin-β receptor is an upstream activator of NF-κB-mediated transcription in melanoma cells. J Biol Chem. 2008;283(22):15399–15408.
60. Xu Y, Zhang C, Wang N, et al. Adiponectin inhibits lymphotoxin-β receptor-mediated NF-κB signaling in human umbilical vein endothelial cells. Biochem Biophys Res Commun. 2011;404(4):1060–1064.
61. Daller B, Müsch W, Röhrl J, et al. Lymphotoxin-β receptor activation by lymphotoxin-α1β2 and LIGHT promotes tumor growth in an NFκB-dependent manner. Int J Cancer. 2011;128(6):1363–1370.
62. Wolf MJ, Seleznik GM, Zeller N, et al. The unexpected role of lymphotoxin β receptor signaling in carcinogenesis: from lymphoid tissue formation to liver and prostate cancer development. Oncogene. 2010;29(36):5006.
63. Fleming JD, Giresi PG, Lindahl-Allen M, et al. STAT3 acts through pre-existing nucleosome-depleted regions bound by FOS during an epigenetic switch linking inflammation to cancer. Epigenetics Chromatin. 2015;8(1):7.
64. Guan Z, Ding C, Du Y, et al. HAF drives the switch of HIF-1α to HIF-2α by activating the NF-κB pathway, leading to malignant behavior of T24 bladder cancer cells. Int J Oncol. 2014;44(2):393–402.
65. Cui X, Kong C, Zhu Y, et al. miR-130b, an onco-miRNA in bladder cancer, is directly regulated by NF-κB and sustains NF-κB activation by decreasing Cylindromatosis expression. Oncotarget. 2016;7(30):48547.
66. Man X, Piao C, Lin X, et al. USP13 functions as a tumor suppressor by blocking the NF-κB-mediated PTEN downregulation in human bladder cancer. J Exp Clin Cancer Res. 2019;38(1):259.
67. Huang Z, Zhong Z, Zhang L, et al. Down-regulation of HMGB1 expression by shRNA constructs inhibits the bioactivity of urothelial carcinoma cell lines via the NF-κB pathway. Sci Rep. 2015;5:12807.
68. Belgorosky D, Langle Y, Mc Cormick BP, et al. Inhibition of nitric oxide is a good therapeutic target for bladder tumors that express iNOS. Nitric Oxide. 2014;36:11–18.
69. Wang WE, Jiang H, Zhu H, et al. Overexpression of high mobility group box 1 and 2 is associated with the progression and angiogenesis of human bladder carcinoma. Oncol Lett. 2013;5(3):884–888.
70. Michaud DS. Chronic inflammation and bladder cancer. Urol Oncol. 2007;25(3):260–268.
71. Ma N, Thanan R, Kobayashi H, et al. Nitrative DNA damage and Oct3/4 expression in urinary bladder cancer with *Schistosoma haematobium* infection. Biochem Biophys Res Commun. 2011;414(2):344–349.
72. Wang DJ, Ratnam NM, Byrd JC, et al. NF-κB functions in tumor initiation by suppressing the surveillance of both innate and adaptive immune cells. Cell Rep. 2014;9(1):90–103.
73. Espinosa L, Cathelin S, D'Altri T, et al. The Notch/Hes1 pathway sustains NF-κB activation through CYLD repression in T cell leukemia. Cancer Cell. 2010;18(3):268–281.
74. Collins PE, Mitxitorena I, Carmody RJ. The ubiquitination of NF-κB subunits in the control of transcription. Cells. 2016;5(2):23.
75. Koksal IT, Yasar D, Dirice E, et al. Differential PTEN protein expression profiles in superficial versus invasive bladder cancers. Urol Int. 2005;75(2):102–106.
76. Massoumi R, Chmielarska K, Hennecke K, et al. Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF-κB signaling. Cell. 2006;125(4):665–677.
77. Gai L, Cai N, Wang L, et al. Ursolic acid induces apoptosis *via* Akt/NF-κB signaling suppression in T24 human bladder cancer cells. Mol Med Rep. 2013;7(5):1673–1677.
78. Deng QF, Sun X, Liang ZF, et al. Cigarette smoke extract induces the proliferation of normal human urothelial cells through the NF-κB pathway. Oncol Rep. 2016;35(5):2665–2672.
79. Geng H, Zhao L, Liang Z, et al. Cigarette smoke extract-induced proliferation of normal human urothelial cells via the MAPK/AP-1 pathway. Oncol Lett. 2017;13(1):469–475.
80. Shah G, Zhang G, Chen F, et al. iNOS expression and NO production contribute to the direct effects of BCG on urothelial carcinoma cell biology. Urol Oncol. 2014;32(1):45.
81. Hosseini a, Koskela LR, Ehrén I, et al. Enhanced formation of nitric oxide in bladder carcinoma in situ and in BCG treated bladder cancer. Nitric Oxide. 2006;15(4):337–343.
82. Ho YS, Chen CH, Wang YJ, et al. Tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces cell proliferation in normal human bronchial epithelial cells through NF-κB activation and cyclin D1 up-regulation. Toxicol Appl Pharmacol. 2005;205(2):133–148.
83. Dalesio O, Schulman CC, Sylvester R, et al. Prognostic factors in superficial bladder tumors. a study of the European Organization for research on treatment of cancer: genitourinary tract cancer cooperative group. J Urol. 1983;129(4):730–733.
84. Oosterlinck W, Kurth KH, Schröder F, et al. A prospective European Organization for research and treatment of cancer genitourinary group randomized trial comparing transurethral resection followed by a single intravesical instillation of epirubicin or water in single stage Ta, T1 papillary carcinoma of the bladder. J Urol. 1993;149(4):749–752.
85. Cote RJ, Datar RH. Therapeutic approaches to bladder cancer: identifying targets and mechanisms. Crit Rev Oncol Hematol. 2003;46:67–83.
86. Lloreta J, Font-Tello a, Juanpere N, et al. FOXO1 down-regulation is associated with worse outcome in bladder cancer and adds significant prognostic information to p53 overexpression. Hum Pathol. 2017;62:222–231.
87. Malats N, Bustos a, Nascimento CM, et al. P53 as a prognostic marker for bladder cancer: a meta-analysis and review. Lancet Oncol. 2005;6(9):678–686.
88. Schmitz-Dräger BJ, Goebell PJ, Ebert T, et al. p53 immunohistochemistry as a prognostic marker in bladder cancer. Eur Urol. 2000;38(6):691–700.
89. Aljabery F, Shabo I, Gimm O, et al. The expression profile of p14, p53 and p21 in tumour cells is associated with disease-specific survival and the outcome of postoperative chemotherapy treatment in muscle-invasive bladder cancer. Urol Oncol. 2018;36(12):530.
90. Konstantakou EG, Voutsinas GE, Karkoulis PK, et al. Human bladder cancer cells undergo cisplatin-induced apoptosis that is associated with p53-dependent and p53-independent responses. Int J Oncol. 2009;35(2):401–416.

91. Haddad JJ, Abdel-Karim NE. NF-κB cellular and molecular regulatory mechanisms and pathways: therapeutic pattern or pseudoregulation? Cell Immunol. 2011;271(1):5–14.
92. Li S, Pinard M, Wang Y, et al. Crosstalk between the TNF and IGF pathways enhances NF-κB activation and signaling in cancer cells. Growth Hormone IGF Res. 2015;25(5):253–261.
93. Alawdi SH, El-Denshary ES, Safar MM, et al. Neuroprotective effect of nanodiamond in Alzheimer's disease rat model: a pivotal role for modulating NF-κB and STAT3 signaling. Mol Neurobiol. 2017;54 (3):1906–1918.
94. Iannetti a, Ledoux AC, Tudhope SJ, et al. Regulation of p53 and Rb links the alternative NF-κB pathway to EZH2 expression and cell senescence. PLoS Genet. 2014;10(9):e1004642.
95. Sun Y, Guan Z, Liang L, et al. NF-κB signaling plays irreplaceable roles in cisplatin-induced bladder cancer chemoresistance and tumor progression. Int J Oncol. 2016;48(1):225–234.
96. Clifford SC, Thomas DJ, Neal DE, et al. Increased mdr1 gene transcript levels in high-grade carcinoma of the bladder determined by quantitative PCR-based assay. Br J Cancer. 1994;69(4):680.
97. Tada Y, Wada M, Migita T, et al. Increased expression of multidrug resistance-associated proteins in bladder cancer during clinical course and drug resistance to doxorubicin. Int J Cancer. 2002;98 (4):630–635.
98. Chen Y, Li L, Zeng J, et al. Twist confers chemoresistance to anthracyclines in bladder cancer through upregulating P-glycoprotein. Chemotherapy. 2012;58(4):264–272.
99. Kausch I, Böhle a. Molecular aspects of bladder cancer: III. Prognostic markers of bladder cancer. Eur Urol. 2002;41(1):15–29.
100. Fondrevelle ME, Kantelip B, Reiter RE, et al. The expression of Twist has an impact on survival in human bladder cancer and is influenced by the smoking status. Urol Oncol. 2009;27(3):268–276.
101. Mitrakas L, Gravas S, Papandreou C, et al. Primary high-grade non-muscle-invasive bladder cancer: high NF-κB expression in tumor specimens distinguishes patients who are at risk for disease progression. Pathol Oncol Res. 2019;25(1):225–231.
    • **Describes the difference in the expression of NF-κB in high risk bladder cancer patients.**
102. Kashiwagi E, Ide H, Inoue S, et al. Androgen receptor activity modulates responses to cisplatin treatment in bladder cancer. Oncotarget. 2016;7(31):49169–49179.
103. Inoue S, Ide H, Mizushima T, et al. Nuclear factor-κB promotes urothelial tumorigenesis and cancer progression via cooperation with androgen receptor signaling. Mol Cancer Ther. 2018;17 (6):1303–1314.
104. Miyamoto H, Yang Z, Chen YT, et al. Promotion of bladder cancer development and progression by androgen receptor signals. J Natl Cancer Inst. 2007;99(7):558–568.
105. Izumi K, Taguri M, Miyamoto H, et al. Androgen deprivation therapy prevents bladder cancer recurrence. Oncotarget. 2014;5 (24):12665.
106. Izumi K, Ito Y, Miyamoto H, et al. Expression of androgen receptor in non-muscle-invasive bladder cancer predicts the preventive effect of androgen deprivation therapy on tumor recurrence. Oncotarget. 2016;7(12):14153.
107. Lee S, Yoon CY, Byun SS, et al. The role of c-FLIP in cisplatin resistance of human bladder cancer cells. J Urol. 2013;189(6):2327–2334.
108. Almeida LO, Abrahao AC, Rosselli-Murai LK, et al. NFκB mediates cisplatin resistance through histone modifications in head and neck squamous cell carcinoma (HNSCC). FEBS Open Bio. 2014;4:96–104.
109. Riemann K, Becker L, Struwe H, et al. Insertion/deletion polymorphism in the promoter of NFKB1 as a potential molecular marker for the risk of recurrence in superficial bladder cancer. Int J Clin Pharmacol Ther. 2007;45(8):423–430.
110. Tang T, Cui S, Deng X, et al. Insertion/deletion polymorphism in the promoter region of NFKB1 gene increases susceptibility for superficial bladder cancer in Chinese. DNA Cell Biol. 2010;29(1):9–12.
111. Ahirwar DK, Mandhani a, Mittal RD. IL-8– 251 T> a polymorphism is associated with bladder cancer susceptibility and outcome after BCG immunotherapy in a northern Indian cohort. Arch Med Res. 2010;41(2):97–103.
112. Yang X, Li P, Tao J, et al. Association between NFKB1– 94ins/del ATTG promoter polymorphism and cancer susceptibility: an updated meta-analysis. Int J Genomics. 2014;2014:1–8.
113. McConnell BB, Vertino PM. TMS1/ASC: the cancer connection. Apoptosis. 2004;9(1):5–18.
114. Ramachandran K, Gordian E, Singal R. 5-azacytidine reverses drug resistance in bladder cancer cells. Anticancer Res. 2011;31 (11):3757–3766.
115. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–674.